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Endothelial Cells and SARS-CoV-2: An Intimate Relationship

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Abstract

Angiotensin-converting enzyme 2 (ACE2) is an important player of the renin-angiotensin-aldosterone system (RAAS) in regulating the conversion of angiotensin II into angiotensin (1-7). While expressed on the surface of human cells, such as lung, heart, kidney, neurons, and endothelial cells (EC), ACE2 is the entry receptor for SARS-CoV-2. Here, we would like to highlight that ACE2 is predominant on the EC membrane. Many of coronavirus disease 2019 (COVID-19) symptoms have been associated with the large recruitment of immune cells, directly affecting EC. Additionally, cytokines, hypoxia, and complement activation can trigger the activation of EC leading to the coagulation cascade. The EC dysfunction plus the inflammation due to SARS-CoV-2 infection may lead to abnormal coagulation, actively participating in thrombo-inflammatory processes resulting in vasculopathy and indicating poor prognosis in patients with COVID-19. Considering the intrinsic relationship between EC and the pathophysiology of SARS-CoV-2, EC-associated therapies such as anticoagulants, fibrinolytic drugs, immunomodulators, and molecular therapies have been proposed. In this review, we will discuss the role of EC in the lung inflammation and edema, in the disseminate coagulation process, ACE2 positive cancer patients, and current and future EC-associated therapies to treat COVID-19.

keywords: endothelial cells; SARS-CoV-2; ACE2; inflammation; coagulation

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1. Introduction

In December 2019, in the city of Wuhan, China, the first cases were reported of a new respiratory infection [1]. Experts determined that the disease, later termed COVID-19, was caused by a novel coronavirus. Since COVID-19 can cause severe acute respiratory distress syndrome (SARS), the new coronavirus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. COVID-19 has since led to the deaths of thousands of people and had caused a serious global impact on both health and economies. Further investigation into the evolution of symptoms in patients with COVID-19 is necessary, since patients may be asymptomatic or have mild, moderate or severe symptoms. Patients with symptoms such as fever, fatigue and cough are considered to be in the mild and moderate phase. Patients with viral pneumonia followed by systemic inflammation are considered to be in the severe phase [2]. And patients with dysfunctional coagulation are considered to be in the critical phase [3]. The SARS-CoV-2 virus can penetrate the host cell by binding to cell surface receptors [4]. As described by Yuki et al (2020) [3], the virus enters the host cell through endocytosis or membrane fusion (penetration), and the viral RNA is then released into the cells and initiates synthesis of viral proteins.

Proteins on the surface of host cells, such as the serine transmembrane protein 2, are important for the initial interaction between viruses and cells [5–10]. Other proteins that can act as viral receptors, such as sialic acid receptors [11,12], the matrix metalloproteinase inducer CD147 [13] and ACE2, then mediate viral entry into the host cell [14]. ACE2, which is part of the RAAS [15,16] is currently the most studied receptor in the context of SARS-CoV-2 [16] and is considered to be important for viral infection [17]. There is evidence that the virus interacts with ACE2 through its spike protein, a trimeric transmembrane glycoprotein that is important for determining viral diversity and host tropism [3,14,15,18]. Experimentally, the spike protein of SARS-CoV-2 has been shown to have high affinity for human ACE2 [7,19]. It is thus possible to use this affinity to estimate the density of ACE2 in different types of tissue [14] and it has been suggested that the density of ACE2 in each tissue may correlate with the severity of disease in that tissue [20–24] .

ACE2 is expressed in several organs, including the lung, heart, kidney and intestine, and is also expressed in EC [25]. Although ACE2 is highly expressed in the

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lung [3,21,26], levels of ACE2 expression in enterocytes are higher than in the lung [27–30]. Based on brain transcriptome data, Chen et al. (2020) [31] reported that, in rats, ACE2 is expressed in the substantia nigra, choroid plexus, non-neuronal cells and many neurons, both excitatory and inhibitory. Expression of ACE2 in the brain has been suggested to contribute to the neurological symptoms associated with COVID-19 [27], as well as the development of neurogenic hypertension [32]. Other studies have indicated the presence of SARS-CoV-2 particles in brain neurons of infected patients [27,33–35]. It has also been suggested that interaction of SARS-CoV-2 with ACE2 in the epithelium of the oral and nasal mucosa leads to the damage reported in these regions [20,27].

Here, we would like to highlight that ACE2 is predominantly expressed in EC [31] , which make up the lining of blood and lymph vessels. Endothelial cells are of fundamental importance in the functioning of the vascular endothelium, which regulates vasodilation, fibrinolysis, aggregation and thrombosis [3,14,36]. ACE2 is present in EC in lung tissue, which represent one third of cells present in the lung [3]. COVID-19 leads to systemic changes in the organization and basic functions of EC, suggesting that these cells are important for the pathophysiology of this disease [2,25,37].

COVID-19 has been associated with extensive recruitment of immune cells, which directly affect EC or act in an immune-mediated manner [25,38]. Inflammatory processes play an important role in the development of COVID-19 and EC are key players in these processes. In this article, we will discuss the role of EC in lung inflammation and edema, disseminated coagulation, and in patients with ACE2-positive cancer. We will also discuss current EC-related therapies to treat COVID-19 and possibilities for the future.

2. Endothelial cells in SARS-CoV-2 infection: inflammation and pulmonary edema

When the immune system generates an effective adaptive response against SARS-CoV-2, the infection can be eliminated and clinical manifestations are absent or may completely disappear [39]. A successful antiviral response is dependent on

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the expression of type I interferons (IFNs) [40–42] and on the activation of T lymphocytes (TCD4$^+$ helper cells (Th) and TCD8$^+$ cytotoxic cells), as well as specific antibody-producing B lymphocytes. If the host organism does not develop an adequate adaptive immune response, the prolongation and amplification of innate mechanisms, combined with dysfunctional adaptive responses, lead to a hyperinflammatory state. The resulting intense and continuous release of pro-inflammatory cytokines underlies the cytokine storm [39] that is characteristic of acute respiratory distress syndrome (ARDS) [39–42], and is responsible for lung damage [39]. Patients with severe COVID-19 have decreased IFN production, as well as aberrant polarization of Th cells (predominantly Th17), increased expression of exhaustion-related surface markers, such as TIM3 and PD-1, and changes in the pattern of cytokine secretion [39,42,43] (Figure 1).

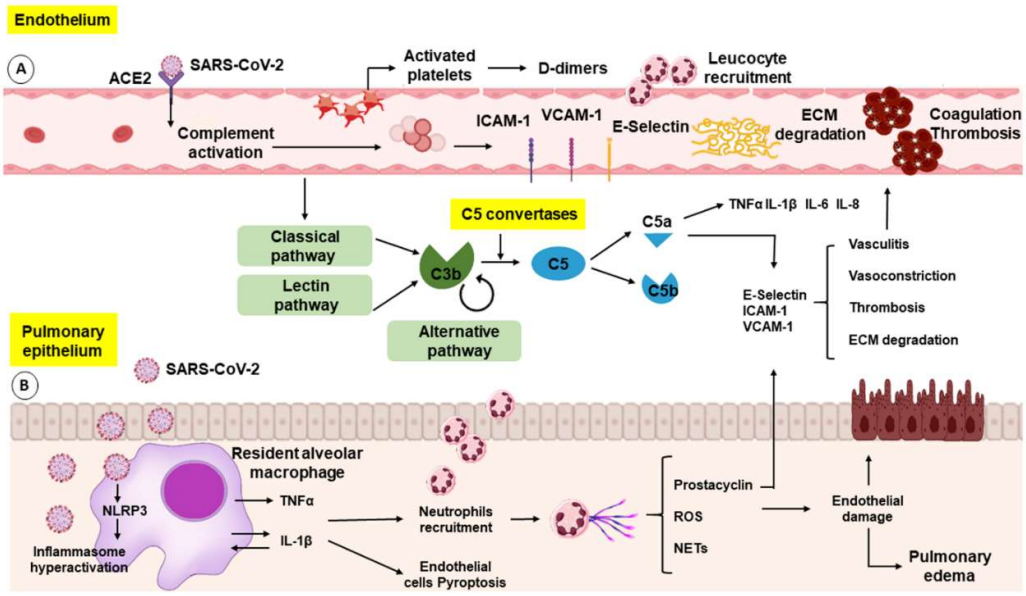

Figure 1. Endothelial cells and pulmonary epithelium in SARS-CoV-2 infection. (A) The binding of SARS-CoV-2 to the ACE2 receptor on endothelial cells leads to the activation of the complement system, a set of plasma proteins with opsonization functions in the immune system. The activation of the complement system induces pro-inflammatory cytokines release (e.g.: TNFα, IL-1β, IL-6, IL-8) and the recruitment of phagocytic cells. The release of these cytokines promotes the activation of the endothelium leading to the expression of selectins (E-selectin and P-selectin) and integrins (ICAM and VCAM) and promote the recruitment of neutrophils and monocytes, causing damage to the endothelium through the release of ROS, production of NETs, degradation of the extracellular matrix (ECM) and more pro-inflammatory cytokines release. The lesion in the endothelium induces the activation of the coagulation cascade and platelet activation leading to coagulation and thrombosis and increasing D-dimers formation. (B) The SARS-CoV-2 infection activates NLRP3, a cytosolic

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receptor in phagocyte cells, leading to the inflammasome, activating caspase 1 and exacerbating the production of IL-1β and TNFα, which in turn, lead to the recruitment of neutrophils, increased production of ROS and NETs, causing endothelial damage, pulmonary edema, and cell death.

SARS-CoV-2 infection is not the primary cause of tissue damage associated with COVID-19. Instead, the damage results from acute hyperinflammation caused by the host immune response, which is mediated by massive release of cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α). These cytokines affect cells of the lung parenchyma, oxygen uptake and EC, and lead to endotheliitis, thrombotic events and intravascular coagulation [44]. The injury caused by SARS-CoV-2 to epithelial cells expressing ACE2 leads to activation of the NLRP3 inflammasome, an acute immune response to the infection, which is directed by alveolar monocytes/macrophages. These cells secrete TNF-α and IL-1β, leading to disseminated activation of NLRP3 and positive feedback that causes cell damage and death, extending to the vasculature and causing leakage and pulmonary edema [45]. TNF-α, generated mainly by activated macrophages, is present at high concentrations in the bronchoalveolar lavage fluid of patients with ARDS and is capable of causing apoptotic death of both epithelial and EC in the lung. Epithelial/endothelial lesions mediated by cytokines/chemokines can impair the integrity of the blood/air barrier, promoting vascular permeability, alveolar edema, leukocyte infiltration (macrophages and neutrophils) and hypoxia [39]. IL-1β, generated by resident macrophages in response to activation of the NLRP3 pattern recognition receptor, plays a role in the recruitment and activation of neutrophils and is a key inducer of neutrophil extracellular traps (NETs). NETs are extracellular structures produced by extrusion of DNA, histones, cytotoxic proteins, myeloperoxidase and cathepsin by neutrophils [46,47]. Uncontrolled production of NETs causes damage to the endothelium, coagulation and thrombosis, and contributes to ARDS [47] .

Large numbers of neutrophils in patients with severe COVID-19 are also associated with high levels of reactive oxygen species (ROS), which activate a cascade of reactions, leading to tissue damage, thrombosis and blood dysfunction [48]. The healthy endothelium produces antithrombotic molecules such as nitric oxide

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(NO) and prostacyclin (PGI2). The endothelial dysfunction observed in COVID-19 patients is related to oxidation and is associated with a decrease in NO levels and an increase in prothrombotic adhesion molecules. Endothelial dysfunction, inflammation, ROS production and increased risk of thromboembolism are intricately linked. ROS stimulate the expression of tissue factor and inhibit protein C, a major anticoagulant [49]. During SARS-CoV-2 infection, activation of the angiotensin 1 receptor also causes increased ROS release, as well as excessive activation of the NLRP3 inflammasome and death of lung EC by pyroptosis [50], as demonstrated experimentally by Ratajczak *et al.* (2020) [51].

Although SARS-CoV-2 primarily infects cells of the pulmonary epithelium, these cells act as a gateway, and alveolar damage is mediated mainly by endothelial damage, which results in activation of cytokines and chemokines and recruitment of cells from the immune system. Acute inflammation in response to SARS-CoV-2 infection also damages EC through overactivation of the complement system, which is involved in intravascular coagulation and thrombosis. The complement system, which consists of a collection of plasma proteins, eliminates microorganisms through opsonization, recruitment of neutrophils and macrophages, generation of proinflammatory mediators, activation of the membrane attack complex and increased humoral immunity. This system is activated by three distinct pathways: the classical, alternative and lectin pathways, which lead to production of C3 and C5. These proteins are subsequently cleaved by their respective convertases, generating the anaphylatoxins, C3a and C5a [44,52–54]. Systemic activation of the complement system via the lectin pathway involves a pattern recognition protein, mannose-binding lectin (MBL) which, together with the MBL-associated serine protease 2 (MASP-2), recognizes mannose residues expressed on various pathogens [52,55], possibly including the SARS-CoV-2 spike protein [52,56]. SARS-CoV-2 binds to its receptor ACE2 on EC and activates first the lectin pathway and then the classical pathway, leading to deposition of C3b. The formation of C3b supports the alternative pathway and participates in formation of the convertases that cleave C5 in the terminal products of the complement system, C5a and C5b-C9 [44]. C5a activates proinflammatory cells, such as neutrophils, and stimulates release of cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, thereby promoting microvascular thrombosis and fibrinolysis. In cells such as neutrophils, EC and platelets, C5a stimulates the

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expression of adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin. These adhesion molecules favor the recruitment, adhesion and transendothelial migration of neutrophils and macrophages, which contributes to destruction of the subendothelial matrix and vasculitis through production of ROS [44,52]. ICAM-1 promotes fibrinogen and fibrin adhesion to EC, which contributes to leukocyte transmigration, endothelial dysfunction and increased risk of thrombus formation and vasoconstriction, when linked to fibrinogen and D-dimer [57]. C5a and the membrane attack complex lead to release of P-selectin and von Willebrand Factor (VWF) by EC, which promotes platelet aggregation and release of thrombomodulin, triggering the coagulation cascade. Finally, C5b-C9 directly promotes platelet activation, leading to platelet aggregation, vascular injury and dysfunction, and coagulation [44]. Histochemical analysis of lung tissues from patients who died from COVID-19 showed deposition of MBL, C3, C4 and C5b-C9, in alveolar epithelial cells, as well as in pneumocytes, inflammatory cells and exudates of alveolar spaces, which indicates activation of the complement system in response to SARS-CoV-2 infection [56].

Activation of EC by SARS-CoV-2 infection thus culminates in pulmonary edema and triggers a coagulation cascade that is implicated in severe cases of COVID-19.

3. SARS-CoV-2 infection: development of thromboembolic, cardiovascular and cerebrovascular complications

Endothelial cells play a crucial role in several physiologic processes, including maintaining homeostasis, blood rheology and vascular barrier function [58]. Physiological and pathological stimulation of ACE2 receptors, which are expressed by ECs in several organs, including the lungs, heart and brain [16,59–61], leads to activation of ECs and subsequent activation of the coagulation cascade [16,62].

Pro-inflammatory cytokines, hypoxia and complement activation can trigger activation of ECs, leading to activation of the coagulation cascade, which depends on interactions between ECs, platelets and coagulation factors [63,64]. Briefly, following stimulation, enhanced fibrin or fibrinogen synthesis activates plasminogen, and plasmin then cleaves the network into soluble fragments, including D-dimer. Binding

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of D-dimer to platelet receptors then leads to the secretion of molecules stored in platelet granules, resulting in platelet activation (Figure 1). Stored VWF and P-selectin are secreted by exocytosis of Weibel–Palade bodies from EC, and VWF tethers platelets and leucocytes to the vessel wall [62,64], leading to platelet aggregation and thrombus formation [44,57,64].

Dysfunction of ECs, together with inflammation caused by SARS-CoV-2 infection, may lead to abnormal coagulation and actively participate in thrombo-inflammatory processes that result in vasculopathy and sepsis, indicating a poor prognosis in patients with COVID-19 [25,58]. D-dimer, a fibrin degradation product that is normally not present in blood unless coagulation has occurred, serves as a biomarker for thrombosis [57] and the presence of D-dimer is associated with poor prognosis and mortality. Laboratory examinations have shown that patients with newly diagnosed SARS-CoV-2 infection have increased levels of D-dimer and that levels of D-dimer increase rapidly as the disease progresse [57,60,65]. Pathological analyses showed that coagulopathy was characterized by high levels of D-dimer, P-selectin and fibrinogen [2,57].

Patients with the severe form of COVID-19 present with a hyperinflammatory state and blood hyperviscosity [65]. Myocardial infarction (MI) [25,64] and stroke have been associated with patients who developed severe COVID-19 and presented with elevated levels of IL-6, D-dimers, troponin and N-terminal pro-brain natriuretic peptide, and erythrocytes [60,65,66], since erythrocyte aggregation represents an important cardiovascular risk factor [67].

Analyzing 68 patients with COVID-19, Goshua et al. (2020) [2] found COVID-19-associated coagulopathy and arterial thrombosis in up to 69% of critically ill patients. In their cohort study, concentrations of D-dimer and thrombin-antithrombin complex were elevated in all patients and were significantly higher in intensive care unit (ICU) patients than in non-ICU patients. VWF parameters were also increased in critically ill patients.

Varga et al. (2020) [25] demonstrated EC involvement across vascular beds of different organs in a post-mortem analysis of patients with COVID-19. Histological analysis showed an accumulation of inflammatory cells associated with the endothelium, as well as apoptotic bodies in the heart, among other organs, revealing lymphocytic endotheliitis and evidence of MI. They found viral elements within EC and an accumulation of inflammatory cells, with evidence of endothelial and

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inflammatory cell death. From these data, they suggest that SARS-CoV-2 infection directly facilitates the induction of endotheliitis in several organs. Autopsies of patients who died from COVID-19 have shown brain hyperemic and edematous tissue and degenerated neurons [60]. Klok and colleagues (2020) [68] observed 184 ICU patients over approximately seven days and found that 31% of these patients demonstrated acute pulmonary embolism, deep-vein thrombosis, ischemic stroke, MI or systemic arterial embolism. Stefanini and Chieffo (2020) [67] analyzed 28 cases of COVID-19 patients who suffered MI in Lombardy, Italy and concluded that MI may represent the first clinical manifestation of COVID-19. Ueki et al. (2020) [69], however, reported a patient presenting with hypercoagulable status, endothelial dysfunction and MI within one week of COVID-19 diagnosis.

In the first detailed report of neurological manifestations of COVID-19 in Wuhan, China, Mao et al. (2020) [60] found neurologic manifestations in 78/218 patients (36%) with SARS-CoV-2 infection treated in their hospital. These manifestations were more common in patients with severe infection than in patients with milder infection. Four of the patients had a stroke and one had a cerebral hemorrhage.

Despite reports showing that older patients and/or patients with comorbidities are at higher risk of complications from COVID-19, it is important to highlight that patients without previous diseases may also present with the most aggressive form of the disease [70]. Deliwala et al. (2020) [24]reported a case of a 31-year-old patient with no risk factors who developed several disease and stroke. Additionally, in two weeks of this pandemic, five patients under the age of 50, diagnosed with SARS-CoV-2 infection in New York City, showed symptoms indicative of onset of ischemic stroke of large vessels. Over the last 12 months, the New York City health service received an average of 0.73 patients under the age of 50 every two weeks with stroke of the large vessels [65]. These authors showed that a diagnosis of cryptogenic stroke was twice as prevalent in COVID-19 positive patients (65.6%), as in COVID-19 negative (30.4%) patients hospitalized in the same hospital system between March 15, 2019 and April 15, 2019. Impressively, these COVID-19 positive patients were younger and had more severe conditions, attributed to the higher prevalence of occlusion of large vessels.

Based on these data, it has been suggested that patients with severe SARS-CoV-2 infection may develop a prothrombotic state, leading to thromboembolic

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complications, and have a greater propensity to develop cardiovascular and cerebrovascular disease [25,60,71].

4) Tumors and COVID-19 risk: Do ACE2 levels matter?

ACE2 is considered to be a regulator of tumor angiogenesis, assisting in the inhibition of angiogenesis in some types of cancer and suppressing signaling through vascular endothelial growth factor (VEGF), VEGFR2 and ERK [4]. Tumor angiogenesis is especially important in tumor progression since it supplies oxygen, nutrients and important factors that increase the invasion and proliferation of tumor cells. It has, therefore, been suggested that expression of ACE2 can cause favorable or unfavorable responses in different tumor types, and further studies are needed to discover how expression affects solid tumors. Chai et al. (2020) [1] have shown the importance of analyzing ACE2 expression in solid tumors. They observed that expression of ACE2 is abnormally high in tumors of the colon, clear renal cells, pancreas, rectum, stomach and lung, whereas expression is decreased in cancer of renal chromophobe cells, testicular germ cells and the thyroid. It has been suggested that changes in ACE2 expression are caused by high levels of DNA methylation and modification of histones [72] or glycosylation [73].

Here, we hypothesize that the level of ACE2 in cancer patients may influence the risk associated with COVID-19 (Figure 2). Patients with comorbidities show an increased risk of developing serious complications when infected with SARS-CoV-2 [74]. Among patients who die from COVID-19, most have comorbidities that influence progression of the virus [75,76] and cancer patients, therefore, require particular attention [77]. Cancer patients infected with SARS-CoV-2 have a high chance of developing serious symptoms, a high risk of mortality and a worse prognosis [1,74,75,78–80]. According to Mehta et al. (2020) [81], the mortality rate associated with COVID-19 is higher in patients with colorectal, lung, breast and prostate cancer. Cancer patients who are receiving treatment, or who have undergone surgery, are also more vulnerable to infection because of their immunosuppressed status and coexisting medical conditions [77,82–87]. The immune status of cancer patients is characterized by overexpression of pro-inflammatory cytokines and a decrease in functional immunosuppressive leukocytes [79]. These characteristics lead to more

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complications when associated with COVID-19, since this is considered to be a hyperinflammatory process [77]. Higher mortality from COVID-19 is expected in patients with cancer compared with other patients. This suggestion is based on laboratory results, which were significantly different in cancer patients and non-cancer patients. The results for cancer patients indicated immune and inflammatory reactions, with high levels of IL-2 and IL-6 receptors, together with possible changes in the prothrombotic state, such as elevation of prothrombin time and erythrocyte sedimentation rate [29,75,79,86].

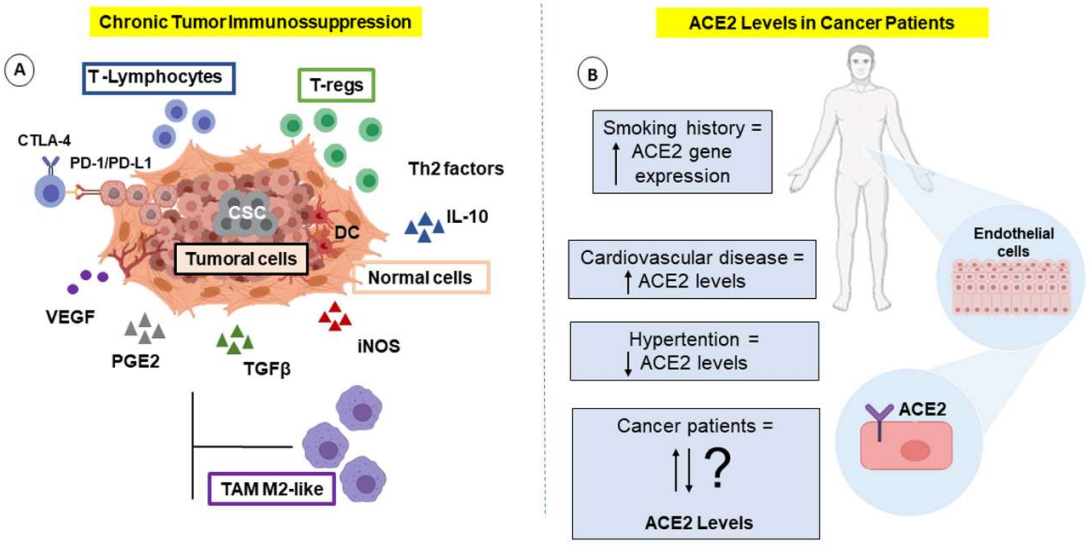

Figure 2. Cancer patient risk to SARS-CoV-2 infection could be influenced by ACE2 levels? (A) Cancer therapeutics and tumor microenvironment might cause immunosuppression in cancer patients. Cancer cells with modified immunogenicity selected immune cells to release immunosuppressive molecules, such as TGFβ, VEGF, PGE2, IL-10, and iNOS, suppressing the proliferation and the cytotoxic response from T-lymphocytes and leading to an anti-inflammatory phenotype (Regulatory T-cells, T-regs). This immunosuppression environment can also induce the recruitment of tumor-associated macrophages with an anti-inflammatory phenotype (TAM-M2) and immature dendritic cells [119]. The chronic immunosuppression in tumor patients can facilitate the infection by SARS-CoV-2 and the COVID-19 severity. (B) Several human disorders can be influenced by levels of ACE2. However, very few are known about ACE2 levels in cancer patients, and this possible increased risk of severity of COVID-19

Ruan et al. (2020) [88] reported that most patients who have died of COVID-19 already had an established disease and that cancer patients are among those at highest risk. In agreement with this, Meng et al. (2020) [75] demonstrated that cancer patients with COVID-19 had a higher risk of mortality (29.4% vs. 10.2%, $P < 0.0001$)

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and Liang et al. (2020) [29] reported that cancer patients had a higher risk of serious events than patients without cancer (39% vs. 8%, P = 0.0003). It has also been shown that smoking, in addition to being an independent risk factor [77], increases the gene expression of ACE2 [79], suggesting greater susceptibility to infection by SARS-CoV-2.

Several human disorders can be influenced by levels of ACE2 [89]. It would be interesting, therefore, to further investigate the correlation between ACE2 levels in patients with solid tumors and the possible increased risk of severity of COVID-19 in this patient cohort.

5. COVID-19 treatment: EC-associated therapies

The unexpected pandemic generated by SARS-CoV-2 has created a global sense of urgency. Current efforts have focused on control and prevention of viral infection, with scientists and physicians responsible for developing protocols, treatments and vaccines. There has been a large global demand for therapies and this demand has been continually increasing in line with the growing number of cases worldwide. Since there has been insufficient time to develop new drugs, health professionals have been challenged to repurpose drugs used to treat other diseases to treat patients with COVID-19. Because of the intrinsic relationship between EC and the pathophysiology of SARS-CoV-2 and, consequently, the development of pathologies such as perivascular inflammation and disseminated intravascular coagulation, treatments with drugs targeting EC have been tested in COVID-19 patients. As examples, anticoagulants have been administered to patients infected with SARS-CoV-2 to decrease coagulopathy, and fibrinolytic drugs, immunomodulators and molecular therapies have also been investigated (Figure 3) [90–93].

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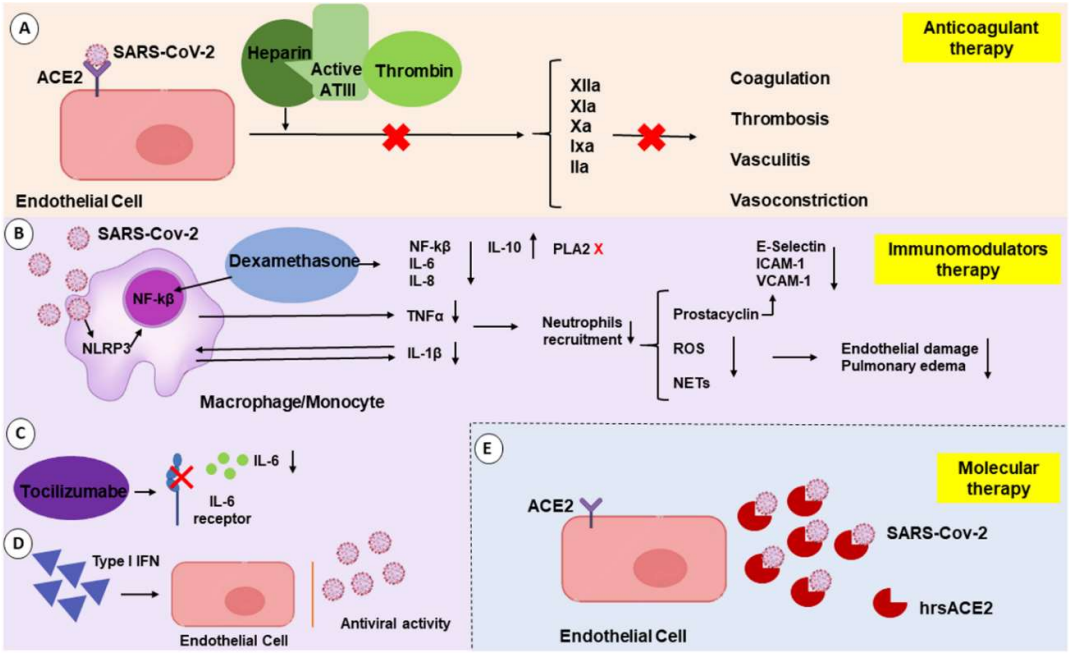

Figure 3. Inflammatory environment triggered by SARS-CoV-2 infection and EC-associated therapeutic models. (A) The binding of heparin to antithrombin 3 (ATIII) increases ATIII activity. This leads to exposure of the ATIII catalytic site, which will interact with thrombin and inhibit its activity. This process leads to the inhibition of important factors for the coagulation process, such as IIa, IXa, Xa, XIa, XIIa. (B) Glucocorticoids such as dexamethasone have an important anti-inflammatory action by modulating inflammatory cytokines and COX-2 and PLA2 activity. Cells from the immune system increase the expression of anti-inflammatory molecules and decrease pro-inflammatory expression. Activation of the glucocorticoid receptor blocks the NFκB gene expression and impairs the expression of proinflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-a. Dexamethasone can bind to Annexin I, which inhibits PLA2 activity impairing the conversion of phospholipids in arachidonic acid, which decreases the production of prostaglandins. (C) Tocilizumab is a monoclonal anti-interleukin-6 (IL-6) antibody which reduces the proinflammatory activity of this cytokine. (D) Type I interferons are a large family of cytokines that mediate innate antiviral immune responses. Type I interferons activate the transcription of several genes giving cells resistance to viral infection (antiviral status). Besides, they promote an increase in the lymphocyte population and its toxicity. (E) Soluble recombinant human ACE2 (hrsACE2) may impair the entry of SARS CoV-2 to the host cell.

5.1) Anticoagulant therapy

Heparin has been of great pharmacological importance for decades because of its role as an anticoagulant and antithrombotic. Heparin is a member of the sulfated linear glycosaminoglycan polysaccharide family and is composed of repeating disaccharide units of uronic acid and D-glucosamine [94]. The anticoagulant action

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of heparin is due to its ability to bind and enhance the activity of coagulation inhibitors, such as the plasma protein antithrombin [95], and this characteristic has shown promise for the treatment of patients hospitalized with COVID-19 once disseminated intravascular coagulation is frequent in these patients. Added to that, long-term bed rest may increase the risk of venous thromboembolism.

In a study carried out in the city of Wuhan, administration of low molecular weight heparin for at least seven days resulted in a lower mortality rate in patients with severe SARS-CoV-2 infection type, i.e., those with elevated D-dimer resulting from fibrin degradation [96]. In addition to its anticoagulant activity, the use of heparin as a treatment has other important benefits. The polyanionic character of heparin allows it to bind to various proteins [95] and enables it to act as an effective inhibitor of viral infection by competing with the virus for binding sites on the cell surface [97] . A recent study confirmed that the S1 subunit of the SARS-CoV-2 spike protein, which contains a receptor-binding domain, interacts with heparin [98]. Heparin can also antagonize histones released by cells damaged by pathogenic infection. There is thus a reduced chance of endothelial injury when generating a "protection" to the endothelium [99,100].

However, studies are needed to determine whether this heparin-mediated endothelial protection would also be beneficial in SARS-Cov-2 infection. There are indications that anticoagulant therapy with heparin may be important to prevent the formation of microthrombin and to treat prothrombotic complications but it is unable to remove advanced clusters of fibrin deposited in the alveolar space in patients with severe infection [101]. In this case, therapies that increase plasminogen activation or downregulate fibrinolytic inhibitors can be used [102].

5.2) Immunomodulators

As discussed above, inflammation is a prominent aspect of SARS-CoV-2 infection, and EC have a unique role in modulating the inflammatory response. As well as harboring established receptors for pro-inflammatory cytokines [38] and playing a role in increased vascular permeability, EC have an intrinsic role in the inflammatory process and in activation of the coagulation cascade in COVID-19 patients. Treatments with immunomodulators, such as dexamethasone, tocilizumab and interferon, have been tested in COVID-19 patients [103,104].

Dexamethasone is an inexpensive and commonly used synthetic adrenal corticosteroid with anti-inflammatory or immunosuppressive properties, depending on whether a low or high dose is administered. Dexamethasone acts as a glucocorticoid agonist and, on binding to the glucocorticoid receptor, inhibits leukocyte infiltration at the site of inflammation and suppresses humoral immune responses [105]. According to the latest results from the RECOVERY trial, in hospitalized patients with COVID-19 receiving invasive mechanical ventilation at randomization and those who were receiving oxygen without invasive mechanical ventilation, the use of dexamethasone for up to 10 days resulted in lower 28-day mortality [106]. Severely ill COVID-19 patients usually have an aberrant inflammatory response and overactivation of the immune system. The correct dose of dexamethasone can, therefore, benefit these patients because of the anti-inflammatory and immunosuppressive effects mentioned earlier. Administration of dexamethasone to patients who were not receiving respiratory support at randomization provided no benefit, and the drug should not be used in this situation. Because of the widespread availability of dexamethasone and the positive clinical evidence, the use of this medication can be indicated in severe clinical cases that require supplemental oxygen or mechanical ventilation.

Tocilizumab is a monoclonal anti-IL-6 receptor antibody, which exerts an immunosuppressive effect by reducing the proinflammatory activity of IL-6. Tocilizumab is used in the treatment of rheumatoid arthritis, systemic juvenile idiopathic arthritis and polyarticular juvenile idiopathic arthritis [107]. Tocilizumab was considered as a possibility for the treatment of SARS-CoV-2 infection because it may soothe the so-called cytokine storm, which, for example, increases endothelial permeability. Scientific studies on the use of tocilizumab are, however, controversial.

In a study carried out in China, a single dose of tocilizumab, administered together with the corticosteroid methylprednisolone, was found to reduce serum levels of IL-6 and C-reactive protein [108]. Although the group claimed that the treatment provided therapeutic benefit, it was suggested that repeated doses of tocilizumab may be more effective in improving the condition of critically ill patients or patients with high levels of IL-6. In another study, administration of tocilizumab provided an improvement in patients' recovery times and a decrease in levels of C-reactive protein. In this study, however, the patients had previously also received hydroxychloroquine and

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azithromycin, so that the concomitant use of these drugs may have influenced the results [109].

Type I IFN is a protein produced and secreted by the immune system after a viral infection, and an impaired type I IFN response has been identified in patients with severe SARS-CoV-2 infection. An excessive inflammatory response and high viral load in the blood have been also observed and may be associated with increased production of cytokines [110,111]. Drugs that increase the amount of IFN have, therefore, been tested as a treatment for COVID-19. It is, however, unclear whether the virus blocks the action of IFNs, or whether people with a deficiency of this protein develop the disease more severely. In a study of 446 patients in China, early administration of IFN reduced mortality, whereas late administration of IFN was associated with increased mortality and delayed recovery [112]. Another group investigated the consequences of treatment with nebulized INF, alone and in combination with the antiviral agent arbidol, in 77 patients. A decrease in the inflammatory biomarker IL-6 and greater viral clearance from the respiratory tract were observed in both cases [113]. There is, therefore, some evidence that the use of IFN as a therapy produces favorable outcomes, but there is still insufficient knowledge to prove its efficacy. It also appears to be important to confirm the ideal stage of the disease at which to administer type I IFN.

5.3) Molecular therapy

As discussed above, the SARS-CoV-2 spike protein binds to ACE2, thereby increasing cell membrane fusion and endocytosis. After activation of the spike protein by a cellular protease (TMPRSS2), ACE2 promotes entry of the virus into the cell [113,114]. Treatments targeting ACE2 have been tested, mainly because this receptor is present in epithelial cells of the respiratory tract, such as type 2 pneumocytes, that are susceptible to SARS-CoV-2 infection [115,116]. One option is the use of recombinant soluble human ACE2 (hrsACE2), which, if present in excess, would intercept the virus-receptor binding and decrease the rate of viral infection [93]. Administration of hrsACE2 has been observed to reduce the infection of capillary and renal organoids by SARS-CoV-2 *in vitro*. As well as acting on the alveolar tract, hrsACE2 may also act on EC and thus prevent viral spread through the bloodstream

[117,118]. This molecular therapy thus has potential in the treatment of SARS-CoV-2 infection.

6) Conclusions

In summary, ACE2 mediates SARS-CoV-2 entry into the host cell and it is a predominant receptor on the EC membrane. In turn, EC has a critical role in the inflammatory and disseminate coagulation process reported in COVID-19 patients, which culminate in a higher risk of thromboembolic, cardiovascular, and cerebrovascular complications in these patients. Several human disorders can be influenced by levels of ACE2. However, ACE2 levels in cancer patients, who have a high chance of developing serious symptoms and a high risk of mortality have not been well explored. Finally, EC-related therapies such as anticoagulants and corticosteroids have been used to treat severe COVID-19 patients and other immunomodulators and molecular therapies have also been studied to this purpose.

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